Effect of Potassium Canrenoate on Cardiac Functions and ($Na^+ + K^+$)-Activated ATPase (37351)

S. I. Baskin, T. Akera, C. R. Puckett, S. L. Brody, and T. M. Brody
(Introduced by J. B. Hook)

Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823

Yeh and co-workers (1, 2) have reported that potassium canrenoate (potassium 3-[3-oxo-17β-hydroxy-4,6-androstadien-17α-yl]propanate) a natriuretic and diuretic aldosterone inhibitor and major metabolite of spironolactone reverses cardiac arrhythmias produced by digitalis. Yeh's studies indicate that potassium canrenoate increases the time to ventricular tachycardia and also abolishes ventricular bigeminy due to ouabain intoxication in dogs. Furthermore, it has been reported that this agent reverses the electrophysiological effects of toxic ouabain concentrations on the canine Purkinje fiber (3). The protective effects could not be attributed to potassium ion.

Since it was observed (2) that potassium canrenoate reverses ouabain-induced arrhythmias in intact dogs but has not been examined in isolated hearts, it is possible that the protection could be mediated reflexly. Studies on the effect of potassium canrenoate on isolated guinea pig heart preparations were therefore undertaken. Further, since it has been proposed that the membrane ($Na^+ + K^+$)-ATPase may be the "receptor" for the inotropic and/or toxic actions of the cardiac glycosides (4), it was of interest to determine whether potassium canrenoate inhibited this enzyme, modified its inhibition produced by the cardiac glycoside or the binding of the cardiac glycoside to, and dissociation from, the enzyme.

Experimental Procedures. For atrial preparations, guinea pigs of either sex weighing 500–700 g were sacrificed, their left atria removed and placed in aerated (95% O_2–5% CO_2) Krebs–Henseleit solution (5) at 35°. The muscle was stimulated with 5 msec pulses from a 161 Tektronix pulse generator at a frequency of 100 beats/min and a voltage not greater than 10% above threshold. Contractile force was estimated in the presence and absence of potassium canrenoate using a Grass FT-03 force-displacement transducer monitored with a recorder. For Langendorff studies, guinea pigs weighing 300–500 g were sacrificed and the hearts perfused by a modified Langendorff method. The effects of potassium canrenoate were investigated either in constant flow (4 ml/min at 27°) or constant pressure (70 mm Hg) systems. Krebs–Henseleit solution (K–H) was the perfusion medium in all studies. In the anti-arrhythmic studies, after a 45 min equilibration period, 1 μM ouabain in K–H solution was perfused until an arrhythmia was observed. The perfusing solution was immediately changed to one containing 1 μM ouabain and a predetermined concentration of potassium canrenoate so that the concentration of the latter in the solution was from 10^{-5} to 10^{-3} M. Contractile force was monitored using a force-displacement transducer (FT-03) attached to the apex of the heart. Heart electrograms and perfusion fluid temperature were continuously monitored in all experiments. Statistical significance was estimated by the use of the Student's t test (6). Potassium canrenoate was generously supplied by G. D. Searle and Co., Chicago, IL.

($Na^+ + K^+$)-ATPase activity of guinea pig heart and rat brain enzyme preparations was assayed according to the methods described by Akera (7) in the presence of 0, 0.05, 0.1, 0.2, 0.5, and 1.0 mM potassium canrenoate. Enzyme activities were also determined in the presence of 0, 0.5, 2.5, or 10 μM ouabain with or without 0.5 mM potassium canrenoate. The concentration of oua-

bain to inhibit 50% of the enzyme activity (I_{50}) was graphically determined for each experiment from a log-probit plot of the data.

For ($Na^+ + K^+$)-ATPase–ouabain binding studies, rat brain enzyme preparations (0.02 mg protein/ml) were incubated with 0.1 μM ^{3}H-ouabain in the presence of 10 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, and 5 mM Tris–ATP for 10 min at 37° with or without 0.5 mM potassium canrenoate. After isolation of the protein and washing, binding was estimated by scintillation counting as previously described (7). For studies on the effect of potassium canrenoate on dissociation of the enzyme ^{3}H-ouabain complex, the ^{3}H-ouabain prebound enzyme was incubated at 37° in the presence of 10 mM NaCl, 7.5 mM KCl, 5 mM $MgCl_2$, 2.5 mM Tris–ATP and 0.1 mM nonlabeled ouabain with or without potassium canrenoate. Aliquots were sampled at 0, 5, 10, 20, and 30 min, after the addition of nonlabeled ouabain to stop the binding reaction, and bound ^{3}H-ouabain estimated as above. The half-time of dissociation ($t_{1/2}$) was calculated from the first-order dissociation constants calculated for each experiment. Statistical significance was determined by the Student's t test (6).

Results. Potassium canrenoate produced a decrease in contractile force in both electrically driven isolated guinea pig left atria and in spontaneously beating Langerdorff preparations. In both preparations, the fall in contractile force was dose-dependent and the effect of several concentrations of potassium canrenoate on contractile force is shown in Fig. 1. In atrial preparations, potassium canrenoate produced decreases in force of 20% and 65% at concentrations of 10^{-4} and 10^{-3} M, respectively. The observed depression in contractile force was not the result of the additional K^+ ion from the drug because increasing the molar concentration of KCl in the perfusing solution by 1 mM from 5.8 to 6.8 mM produced only a modest fall in contractile force in control hearts. No effect was seen when less than 1 mM KCl was added to the control perfusion solution. In the spontaneously-beating Langendorff preparations 10^{-4} M potassium canrenoate produced a 20–40% increase in coronary flow and concentrations of potassium canrenoate as low as

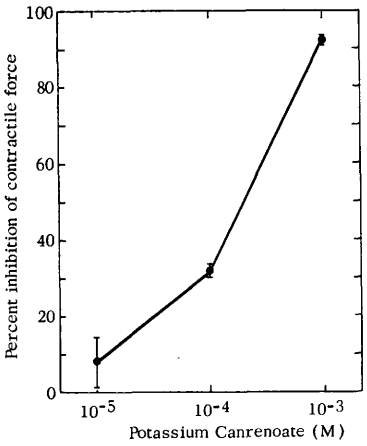

FIG. 1. The effect of potassium canrenoate on contractile force in the Langendorff preparation of guinea pig heart. Mean of 4 experiments. Vertical lines indicate SEM.

10^{-5} M increased heart rate by approximately 20%.

Figure 2 shows the effects of two potassium canrenoate concentrations on ouabain-induced arrhythmias in the spontaneously beating guinea pig heart. In Panel A is shown the lack of response of 1×10^{-4} M potassium canrenoate added to the perfusion medium following the genesis of arrhythmias by ouabain. A significant positive inotropic effect was observed at 14 min and arrhythmias supervened at 19 min after the beginning of the ouabain perfusion. Potassium canrenoate failed to reverse the glycoside toxicity. There also was some decrease in contractile force. Only at high canrenoate concentrations (10^{-3} M) was it possible to abolish the arrhythmia (Panel B). However, it is likely that this effect is due to potassium ion since the additional 1 mM KCl in the perfusion medium (KCl concentration increased from 5.8 to 6.8 mM) also converted the ouabain-induced arrhythmia to a normal rhythm (Panel C). At high potassium canrenoate concentrations (1 mM) reversal of toxic glycoside effects are not observed if the K^+ concentration in the perfusion medium is 2.8 mM (unpublished observations). The experiments in Fig. 2 are typical and at least four experiments were performed in each group.

The ability of potassium canrenoate to

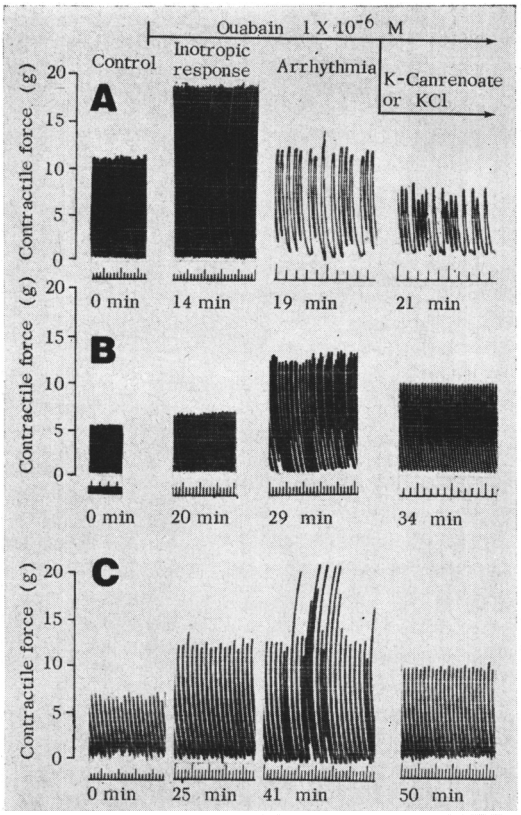

FIG. 2. The effect of potassium canrenoate and potassium chloride on ouabain-induced arrhythmias in the Langendorff preparations of guinea pig heart. Panels A and B: Potassium canrenoate 1×10^{-4} *M* and 1×10^{-3} *M*, respectively. Panel C; Potassium chloride 1×10^{-3} *M* (final concentration of KCl: 6.8 m*M*). Typical tracings. At least 4 experiments performed in each group.

affect the interaction between the cardiac glycoside, ouabain, and $(Na^+ + K^+)$-ATPase was extensively studied. Potassium canrenoate itself failed to inhibit the activity of enzyme preparations from either guinea pig heart or rat brain. Control $(Na^+ + K^+)$-ATPase was 230 ± 17 μmoles ATP hydrolyzed per milligram enzyme protein in 60 min and in the presence of 1 m*M* potassium canrenoate the value was 215 ± 12 (mean ± SEM of 4 determinations). The effects on the ouabain-$(Na^+ + K^+)$-ATPase interaction were also negative (Table I). Potassium canrenoate modified neither the rate of binding of ^{3}H-ouabain to the enzyme (Table I, column 1) nor its rate of dissociation when the glycoside and enzyme were prebound and then allowed to dissociate (Table I, column 2). Furthermore, the ouabain-induced inhibition of $(Na^+ + K^+)$-ATPase was unchanged by the addition of 5×10^{-4} *M* potassium canrenoate as no difference in the I_{50} was observed (Table I, column 3). It would appear that if potassium canrenoate reduces the toxicity of the cardiac glycosides, the effect is independent of an action on $(Na^+ + K^+)$-ATPase.

Discussion. It is possible to reconcile the differences between the present studies and those of Yeh and co-workers (1–3). While it may be difficult to attribute the disparity in the findings to species differences, it is conceivable that the successful reversal of glycoside arrhythmias by potassium canrenoate in the intact and open-chest dog may be attributed to the intact autonomic innervation in these preparations compared to the absence of nervous control in the guinea pig atrial and Langendorff systems. These data lead to the conclusion that the abolition of the digitalis-induced arrhythmias by potassium canrenoate is a consequence of a yet unknown action of the drug on the autonomic innervation to the heart.

In a similar vein, our finding that relatively

TABLE I. Effect of Potassium Canrenoate on Ouabain-$(Na^+ + K^+)$-ATPase Interaction.

	^{3}H-ouabain binding (pmole/mg protein)	$t_{1/2}$ for dissociation (min)	I_{50} for ouabain inhibition of $(Na^+ + K^+)$-ATPase (μ*M*)
KCl 0.5 m*M*[a]	59.4 ± 3.2	38.7 ± 1.6	2.59 ± 0.43
K-Canrenoate 0.5 m*M*	55.1 ± 3.9[b]	34.1 ± 1.5[b]	2.54 ± 0.10[b]

[a] KCl added to serve as control for potassium canrenoate.

[b] Not significantly different from KCl control. Four animals were used in each group in all studies.

low concentrations of potassium canrenoate cause an increase in rate and a decrease in contractile force in the isolated heart are not inconsistent with the failure to observe such changes in the intact animal. Reflex phenomena can normalize such effects in an animal with an intact nervous system. Moreover, negative inotropic responses are more readily observed in denervated, decompensated preparations like the isolated Langendorff preparation.

The failure of potassium canrenoate to alter the inhibitory action of the cardiac glycosides on $(Na^+ + K^+)$-ATPase, to inhibit the enzyme or to modify the glycoside binding to, and dissociation from, the enzyme would tend to rule out this transport enzyme as a site for the antiarrhythmic action of potassium canrenoate.

Summary. Potassium canrenoate in low concentrations caused a negative inotropic effect, increased heart rate and increased coronary flow in isolated guinea pig hearts. It failed to modify ouabain-induced arrhythmias in Langendorff preparations except at very high concentrations where the effect could be due to K^+ ion. Potassium canrenoate did not inhibit $(Na^+ + K^+)$-ATPase nor did it modify the ouabain-enzyme interaction.

Supported by a grant from the Michigan Heart Association.

1. Yeh, B. K., and Sung, P. K., Eur. J. Pharmacol. **19**, 155 (1972).

2. Yeh, B. K., Sung, P. K., and Saha, A. K., Circ. Res. **31**, 915 (1972).

3. Yeh, B. K., and Lazzara, R., Pharmacologist **13**, 224 (1971).

4. Lee, K. S., and Klaus, W., Pharmacol. Rev. **23**, 193 (1971).

5. Winegrad, S., and Shanes, A. M., J. Gen. Physiol. **45**, 371 (1962).

6. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).

7. Akera, T., Baskin, S. I., Tobin, T., and Brody, T. M., Naunyn-Schmiedeberg's Arch. Pharmakol. **277**, 151 (1973).

Received Feb. 12, 1973. P.S.E.B.M., 1973, Vol. 143.